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LACTATE AND GLYCOGEN METABOLISM IN THE LIZARD *DIPSOSAURUS DORSALIS* FOLLOWING EXHAUSTIVE EXERCISE

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Summary

We evaluated the metabolic mechanisms by which the iguanid lizard *Dipsosaurus dorsalis* deals with the lactate which accumulates during vigorous exercise. Fasted, cannulated lizards were run for 5 min on a treadmill at 40°C, which elevated whole-body lactate to 24 mmol l^{-1} and depleted hindlimb glycogen to 70% of resting levels. Oxygen consumption increased fivefold and respiratory exchange ratios approached 2·0. Exhausted animals were then injected intravenously with either [U-^{14}C]lactate or [U-^{14}C]glucose, and allowed to recover quietly on the treadmill at 40°C. After 2 h, 79% of the accumulated lactate had been removed and hindlimb muscle glycogen stores had returned to pre-exercise levels. Although blood glucose remained unchanged at $8{\cdot}6 \pm 0{\cdot}27$ mmol l^{-1} throughout the recovery period, whole-body glucose increased significantly from $1{\cdot}6 \pm 0{\cdot}23$ to $5{\cdot}5 \pm 0{\cdot}38$ mmol l^{-1} ($P < 0{\cdot}05$). Based on isotope distribution, 50% of the lactate removed was used to synthesize glucose and glycogen, but only 16% of the lactate was oxidized. Lactate oxidation accounted for about 40% of the post-exercise oxygen consumption. Lactate rather than glucose appeared to be the prevalent substrate for muscle glycogen synthesis under these conditions. These animals appear to employ a strategy of lactate removal which is different from that in mammals; favoring lactate-supported gluco- and glyconeogenesis and rapid muscle glycogen replenishment instead of rapid lactate removal *via* oxidative pathways.

Introduction

Reptiles and amphibians are noted for their capacity to support vigorous activity by mechanisms of anaerobic energy metabolism. Several studies have documented that small amphibians and reptiles will employ anaerobic glycolysis to produce a far greater fraction of the total ATP utilized during brief exercise than do comparable-sized mammals (Bennett, 1978). In addition to causing significant ventilatory and acid–base disruption, this metabolic strategy results in substantial

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muscle glycogen depletion and lactic acid accumulation. Glycogen depletion and lactate accumulation are correlated with exhaustion in reptiles and amphibians, as they are in other vertebrates, and limited data suggest that the duration or intensity of subsequent activity is constrained during the period when these metabolic disturbances are thought to persist.

The mechanisms by which reptiles and amphibians remove the accumulated lactate and replace glycogen stores are not known. In rats and dogs, the principal fate of post-exercise lactate is oxidation, with gluconeogenic removal accounting for no more than 20% of the lactate (Brooks *et al.* 1973; Issekutz *et al.* 1976). Although one study indicates that gluconeogenesis may be the principal pathway for lactate removal in man (Hermansen & Vaage, 1977), it has been criticized for technical reasons and is at odds with other studies of human lactate metabolism (Jorfeldt, 1970; Mazzeo *et al.* 1986; Peters-Futre *et al.* 1987). One might anticipate that lower vertebrates, which rely more heavily on glycogen degradation during activity, might employ a different strategy for lactate removal and glycogen replenishment. There are some data to suggest that this indeed may be the case. Studies have shown that glycogen replenishment and lactate removal are temporally linked in reptiles to a greater extent than in mammals (Gratz & Hutchison, 1977; Gleeson, 1982). This temporal linkage would be consistent with a mechanism of lactate removal where a significant fraction of the lactate accumulated during activity is conserved and utilized to resynthesize muscle glycogen *via* gluconeogenic pathways. Additional evidence for lactate-supported glycogen replenishment comes from *in vitro* experiments. Isolated muscles from frog and lizard have been shown to be capable of significant rates of glycogenesis from lactate at rates higher than those in mammals (Bendall & Taylor, 1970; Connett, 1979; Gleeson, 1985).

This is the second of a series of papers that investigates the mechanisms of lactate removal and glycogen synthesis following exercise in a small iguanid lizard. Previously, we have shown that red muscle of the lizard *Dipsosaurus dorsalis* is capable of synthesizing glycogen from lactate *in vitro* (Gleeson, 1985). In this paper, we report the responses of the intact animal to 5 min of vigorous activity. We detail the kinetics of lactate removal and glycogen resynthesis *in vivo* during recovery, and address the fates of the post-exercise lactate.

Materials and methods

Lizards (*Dipsosaurus dorsalis*) were collected from the low desert of California near Cathedral City (Riverside Co.), under California Fish and Game permit no. 807, in July 1987. Lizards were transported to Boulder, CO, where they were segregated by sex and kept in photothermal-equipped cages with water available *ad libitum*. Animals were fed three times weekly on a diet of shredded lettuce sprinkled with a powdered mixture of rat chow, Kellogg's Special K breakfast cereal and multi-vitamins. Animals held under these conditions gain weight and become seasonally reproductive if given the opportunity.

Animals were anesthetized (Nembutal, 37·5 $\mu g\,g^{-1}$) 4–6 days prior to experimentation and fitted with a right jugular cannula fashioned from PE 10 and PE 50 polyethylene tubing. Pre- and immediate post-exercise animals were sham-operated 4–6 days prior to experimentation. Animals recovered from anesthesia at 38°C, and were then held singly in photothermally equipped cages with water but no food until experimentation.

Each lizard was fitted with a loose-fitting, lightweight clear acetate mask from which expired gases were drawn by vacuum. The lizard was then placed within a cloth-covered container and the container placed onto the surface of a motor-driven treadmill maintained at 40 ± 1°C. After sufficient time had passed for a stable metabolic state to be reached (2–4 h), animals were removed from the container and forced to run on the treadmill. Animals were encouraged to run by prodding the tail and hindlimbs. Treadmill speed was adjusted continuously to elicit maximal locomotor activity for each individual. Locomotor activity was initially vigorous (1·5–3·0 $km\,h^{-1}$) and continuous, becoming progressively slower and finally intermittent in most animals after about 3·5 min. All animals were exhausted and unable to locomote after 5 min, when all attempts to stimulate the animal ceased. Animals were then infused with 50 μl of 0·9 % NaCl containing either 3 μCi [U-^{14}C]-L-lactate or [U-^{14}C]-D-glucose through the jugular cannula. The cannula was then flushed with 200 μl of saline and the animal returned to the container on the treadmill surface where it was left covered and undisturbed for 30–120 min.

Gas exchange was monitored continuously before, during and after exercise. Fractional concentrations of oxygen and carbon dioxide were measured in the air downstream from the mask with an Applied Electrochemistry S3A O_2 analyzer and an Anarad AR 411 CO_2 analyzer, respectively. Airflow from the mask was controlled and monitored with a Tylan mass flowmeter. Analog outputs from gas analyzers and flowmeter were digitized by an Interactive Structures AI13 A/D board and an Apple IIe which computed averaged rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) and respiratory exchange ratio (R, $\dot{V}_{CO_2}/\dot{V}_{O_2}$) every 60 s according to previously published steady-state equations (Withers, 1977).

All air drawn from the mask during the recovery period was bubbled through CO_2 traps downstream from the gas analyzers. Each trap contained 25 ml of an ethanolamine–methylcellusolve (2:1) solution which had a CO_2 capture efficiency of 97 %. Airflow was transferred to a new trap after 5 min of recovery, again after an additional 10 min, and then every 15 min thereafter. Radioactivity (^{14}C) was determined in a 1·0 ml sample of the trap solution mixed with 2·0 ml of Scintiverse™ cocktail.

After recovery in the treadmill container, animals were quickly removed, the mask taken off, and the lizard decapitated. Hindlimbs were frozen *in situ* with liquid-N_2-cooled tongs, separated from the carcass and placed into a liquid N_2 reservoir. A 200 μl blood sample was taken from the neck wound and mixed with 800 μl of 0·6 $mol\,l^{-1}$ $HClO_4$, the liver removed, blotted and frozen between tongs.

The remaining carcass was then weighed and homogenized in approximately 5 vol of cold 0·6 mol l^{-1} $HClO_4$ for 30 min while evolved gases were drawn from the homogenization vessel and passed through a CO_2 trap. Homogenization was interrupted after the first 2 min and a 3 ml sample removed for later analysis of carcass metabolites. Protein in the $HClO_4$ precipitate was measured colorimetrically using Biuret reagent (Sigma 540-2) and bovine serum albumin standards. All tissues and homogenates were stored at −75 °C until analysis.

Glycogen was isolated from 0·5 μl samples of tissue–$HClO_4$ homogenates according to Hassid & Abraham (1957). Recovery of added glycogen by this procedure was found to be better than 99 %. The final, washed glycogen pellet was solubilized in 0·5 ml (1·0 ml, liver) of water. A 100 μl sample was added to 3 ml of Scintiverse cocktail and counted for ^{14}C activity in a Beckman LS 3133T scintillation counter, calibrated with both internal and external standards. All samples were corrected for quench. The remaining solubilized glycogen sample was diluted 2:1 with water and glycogen measured by reaction of 100 μl samples with 3 ml of anthrone reagent incubated at 90 °C for 15 min. Absorbance of the cooled reaction mixture was measured at 620 nm and compared with standard curves to calculate glycogen concentration.

Blood and tissue glucose and lactate were separated for determination of their specific activities by high performance liquid chromatography (HPLC). Perchlorate supernatants were neutralized with 10 mmol l^{-1} K_2CO_3 and centrifuged to remove perchlorate salts. The HPLC system consisted of an LDC/Milton Roy CM4000 multiple-solvent pump, SM4000 programmable wavelength detector set at 195 nm, and a CI-10B integrator, all controlled by an IBM XT computer and LDC/Milton Roy software. The mobile phase consisted of 0·008 mol l^{-1} H_2SO_4 pumped at 0·6 ml min^{-1} through a Bio-Rad cation–H^+ precolumn and Aminex HPX-87H 300 mm × 7·5 mm column maintained at 25 °C. Metabolite peaks were identified and calibrated by injection of known standards. Samples (20 μl) of the neutralized supernatants were chromatographed and fractions were collected and counted for ^{14}C. Preliminary studies showed that detectable ^{14}C was present only in fractions corresponding to glucose and lactate.

Blood glucose and lactate concentrations were calculated from integrated areas under the chromatogram peaks. Tissue glucose was measured colorimetrically using the glucose oxidase reaction according to Bergmeyer & Bernt (1974). Tissue [lactate] was measured enzymatically according to Gleeson (1985).

Whole-body metabolite concentrations ([X]) were calculated as a weighted average from carcass, hindlimb and liver concentrations as follows:

$$\{([X]_{carc} \times M_{carc}) + ([X]_{liv} \times M_{liv}) + ([X]_{hdlb} \times 2M_{hdlb})\}/(M_{carc} + M_{liv} + 2M_{hdlb}) = [X]_{animal},$$

where M equals tissue mass. Analyzed tissues averaged 86·5 ± 0·01 % of total body mass. All statistics were performed using StatWorks™ programs (Cricket, Inc) written for the MacIntosh+ computer. All data are reported as ±1 S.E.M.

Results

Dipsosaurus dorsalis showed physiological responses to exercise similar to those of other reptiles. Oxygen consumption ($\dot{V}_{O_2}$) during 5 min of treadmill exercise averaged $0{\cdot}6 \pm 0.02$ ml $O_2\,g^{-1}\,h^{-1}$, four times greater than during the period just prior to exercise (Fig. 1). Peak $\dot{V}_{O_2}$ occurred 2–3 min into exercise and averaged $0{\cdot}9 \pm 0{\cdot}03$ ml $O_2\,g^{-1}\,h^{-1}$. Carbon dioxide production ($\dot{V}_{CO_2}$) rose rapidly during exercise and averaged $1{\cdot}1 \pm 0{\cdot}04$ ml $CO_2\,g^{-1}\,h^{-1}$ during the 5 min exercise period. Carbon dioxide production remained elevated relative to the rate of oxygen consumption for the first 15–40 min of quiet recovery. The ratio R ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) was maximal (R = 1·99) 5 min after cessation of exercise, reached minimal values of 0·3–0·4 after 90 min of recovery, and then slowly rose towards pre-exercise values of $0{\cdot}53 \pm 0{\cdot}02$ (Fig. 1). A pre-exercise value of R = 0·53 reflects persistent CO_2 retention following stress associated with handling (Gleeson & Bennett, 1982). After 120 min of recovery, $\dot{V}_{O_2}$ was still elevated relative to pre-exercise rates, although $\dot{V}_{CO_2}$ had returned to rates not significantly different from pre-exercise after 90 min ($P > 0{\cdot}05$ *t*-tests).

Blood and whole-animal lactate concentrations were elevated during exercise by 11- and 2·8-fold, respectively (Fig. 2). Peak tissue lactate concentration occurred in hindlimb muscle, where it exceeded 30 mmol kg^{-1} immediately after exercise (Table 1). Hindlimb [lactate] was not significantly different from blood [lactate] after 30 min. Approximately 48% of the lactate accumulated during exercise was removed after 60 min, and approximately 79% after 2 h. Net whole-

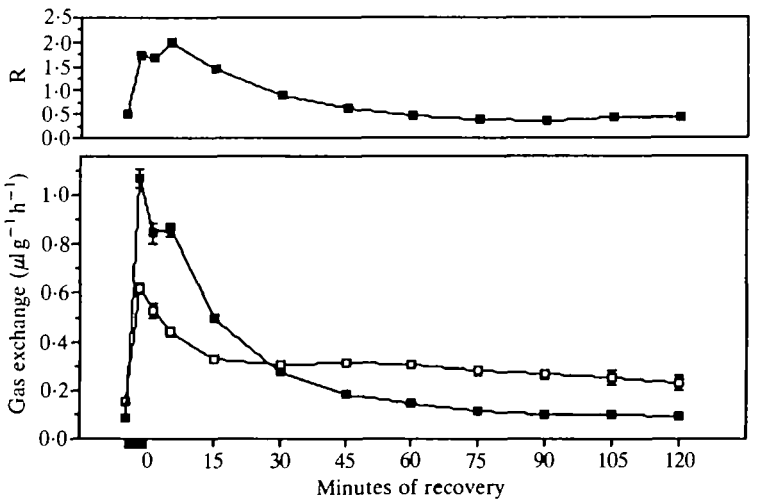

Fig. 1. Metabolic gas exchange following 5 min of exhaustive treadmill exercise in the lizard *Dipsosaurus dorsalis* at 40°C. One-minute averages were combined to provide averages over 5- to 15-min intervals. Upper panel, respiratory exchange ratio, lower panel, oxygen consumption (□) and carbon dioxide production (■). Sample size varied between 12 and 52, error bars represent ±1 S.E.M., and are sometimes obscured by the symbol. Bar along abscissa indicates period of treadmill exercise.

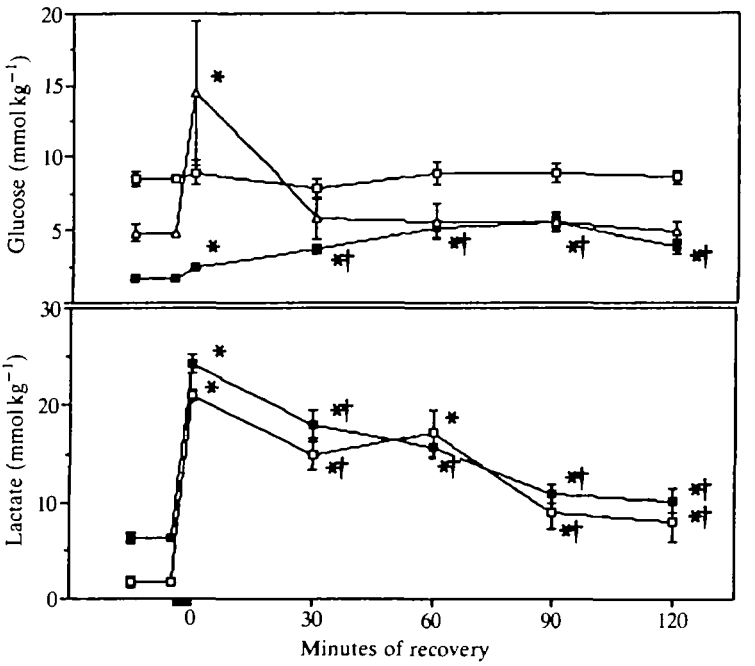

Fig. 2. Tissue glucose and lactate concentrations following 5 min of exercise in the lizard *Dipsosaurus dorsalis*. Upper panel, glucose changes in whole blood (□), liver (△) and whole animal (■). Lower panel, lactate changes in blood and in the whole animal following exercise. Each symbol represents mean ±1 S.E.M. of 8 or 9 animals. Asterisks indicate significant difference ($P<0.05$) from resting values, daggers indicate significant difference from time 0 post-exercise values.

body lactate removal proceeded at a mean rate of 0·12 mmol kg^{-1} min^{-1}. Whole-animal free glucose levels increased through the first 90 min of recovery, and then began to decline (Table 1, Fig. 2). This elevation was not due to changes in blood glucose levels, which averaged 8·6 ± 0·27 mmol l^{-1} ($N = 51$) and did not change during or after exercise (ANOVA, $P = 0.8$). Liver free [glucose] increased considerably immediately after exercise (Fig. 2), but returned to resting levels after 30 min of recovery.

Five minutes of exhaustive exercise rapidly reduced hindlimb muscle glycogen stores to approximately 70 % of resting levels (Fig. 3). Hindlimb glycogen was then replaced during the next 2 h. Hindlimb glycogen levels after 60 min were significantly ($P<0.05$) greater than at the end of exercise, but were not significantly different from that in resting animals after 30 min. Carcass [glycogen] did not change significantly ($P>0.05$) following exercise, so that calculated whole-animal glycogen concentrations changed very little over the same period. Whole-animal [glycogen] was significantly different from resting levels only at 90 min (Fig. 3), owing to the contribution of elevated liver glycogen concentrations measured at that time (Table 1).

Table 1. *Metabolite concentrations following exhaustive exercise in the lizard* Dipsosaurus dorsalis

Time (min)	Lactate			Glucose		Glycogen		
	Animal	Hindlimb	Blood	Animal	Blood	Animal	Hindlimb	Liver
Rest	6·4 ± 0·57	6·7 ± 0·66	1·8 ± 0·53	1·6 ± 0·23	8·50 ± 0·484	47·6 ± 5·29	46·8 ± 4·35	384 ± 63·7
0	24·2 ± 0·92*	30·9 ± 0·93*	21·1 ± 0·56*	2·4 ± 0·21	8·96 ± 0·874	47·8 ± 5·93	32·8 ± 4·30*	501 ± 64·6
30	18·0 ± 1·49*†	20·4 ± 2·19*†	14·9 ± 1·42*†	3·7 ± 0·34*†	7·82 ± 0·735	47·6 ± 4·22	38·2 ± 3·47	501 ± 75·7
60	15·7 ± 1·13*†	13·7 ± 1·48*†	17·1 ± 2·40*	5·1 ± 0·58*†	8·93 ± 0·751	54·8 ± 5·52	55·4 ± 4·31†	468 ± 71·6
90	10·9 ± 0·96*†	9·5 ± 0·91*†	8·9 ± 1·56*†	5·5 ± 0·38*†	8·94 ± 0·669	68·5 ± 4·81*	52·5 ± 4·06†	716 ± 43·3*
120	10·2 ± 1·29*†	8·2 ± 0·98*	8·0 ± 2·13*†	3·9 ± 0·55*†	8·59 ± 0·468	60·5 ± 6·73	58·2 ± 6·98†	401 ± 68·5

* Different from rest, $P < 0·05$; † different from post-exercise, $P < 0·05$.
Metabolite concentrations are given in mmol kg^{-1}.
Values are mean ± S.E.M., $N = 8$ or 9.

Table 2. *Metabolite specific activities (μCi $mmol^{-1}$) during recovery in* Dipsosaurus dorsalis *following [^{14}C]lactate or [^{14}C]glucose injection*

Time (min)	Glucose				Lactate				Glycogen					
	Blood	*Blood*	Carc	*Carc*	Blood	*Blood*	Carc	*Carc*	Carc	*Carc*	Hdlb	*Hdlb*	Liver	*Liver*
30	0·98	–	0·66	–	5·05	–	1·94	–	0·11	–	0·15	–	0·007	–
	±0·153	–	±0·107	–	±1·08	–	±0·126	–	±0·022	–	±0·054	–	±0·0017	–
60	1·54	*10·6*	1·01	*7·94*	2·84	*0·32*	1·84	*0·064*	0·14	*0·14*	0·26	*0·20*	0·056	*0·14*
	±0·405	*±1·20*	±0·162	*±1·30*	±0·357	*±0·087*	±0·157	*±0·014*	±0·018	*±0·312*	±0·047	*±0·065*	±0·0241	*±0·042*
90	1·44	–	1·00	–	3·06	–	1·58	–	0·27	–	0·47	–	0·068	–
	±0·229	–	±0·133	–	±0·519	–	±0·163	–	±0·038	–	±0·075	–	±0·0409	–
120	2·28	*10·3*	2·90	*6·94*	3·69	*0·58*	1·60	*0·068*	0·30	*0·23*	0·49	*0·68*	0·29	*0·23*
	±0·575	*±0·84*	±1·13	*±0·749*	±0·519	*±0·097*	±0·163	*±0·014*	±0·040	*±0·030*	±0·063	*±0·091*	±0·107	*±0·052*

* Specific activities following [^{14}C]glucose injection are given in italics.
Carc, carcass; hdlb, hindlimb.
Values are mean ± S.E.M., $N = 8$ or 9.

Metabolite specific activities (SA) during recovery are summarized in Table 2 for animals injected with [U-^{14}C]lactate or [U-^{14}C]glucose following exercise. Hindlimb glycogen SA increased linearly during the first 90 min of recovery, and did not change significantly thereafter. In contrast, liver glycogen SA remained low during the initial 90 min, and increased significantly only during the last 30 min of the recovery period.

Fig. 4 summarizes the fate of the ^{14}C injected as [^{14}C]-L-lactate following vigorous exercise. These data were calculated from metabolite concentrations and

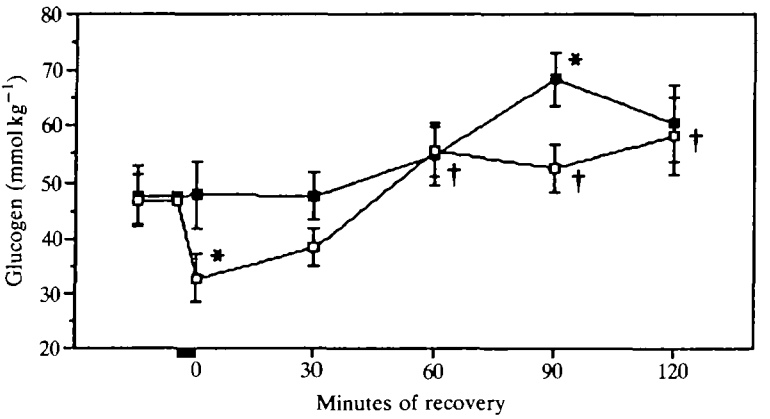

Fig. 3. Glycogen changes following 5 min of exercise in the lizard *Dipsosaurus dorsalis* in hindlimb (□) and in the whole animal (■). Each symbol represents mean ±1 S.E.M. of 8 or 9 animals. Asterisks indicate significant difference ($P<0{\cdot}05$) from resting values, daggers significant difference from time 0 post-exercise mean value.

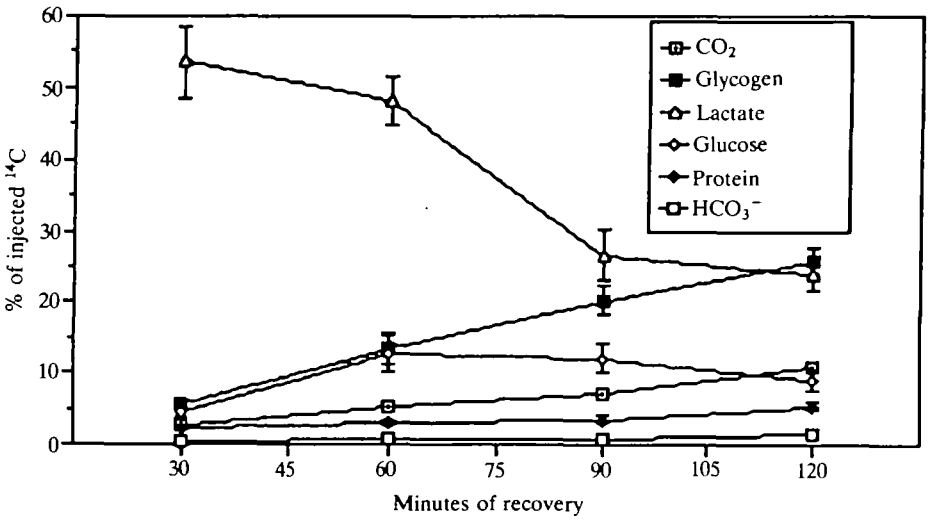

Fig. 4. Distribution of ^{14}C label in *Dipsosaurus dorsalis* as a function of time following intravenous injection of [U-^{14}C]-L-lactate after 5 min of exhaustive exercise at 40°C. Each symbol represents mean ±1 S.E.M. of 8 or 9 animals.

specific activities at each time interval, and from the activity of the ^{14}C collected in CO_2 traps. The whole-body lactate pool represented the largest single site of the injected label during all but the last sampling period. Even after 2 h of recovery, 24 % of the injected label remained within the lactate pool. Incorporation of lactate carbon into tissue glycogen increased steadily from 6 % after 30 min of recovery to 26 % after 2 h. Respiratory CO_2 was the fate of an additional 10·7 % of the injected lactate carbon after 2 h. Labeled carbon in tissue bicarbonate accounted for an additional 1·4 % after 2 h, while protein contained 5·4 % of the label. The free glucose pool accounted for a maximum of 12·5 % after 60 min. Although the glucose SA continued to rise during the last 60 min of recovery, the smaller glucose pool after 120 min of recovery accounted for only 9 % of the label. Label in these six metabolites accounted for an average of 75 % of the injected label and ranged between 69 % (at 30 min) and 83 % (60 min). The remaining label was presumably distributed among several other intermediates at activities too low to be quantified following HPLC separation.

Discussion

Metabolic responses to exercise

The principal mechanism for lactate removal following exercise in *Dipsosaurus dorsalis* is gluconeogenic conversion to skeletal muscle glycogen. Although the pathways involved do not seem remarkable, the strategy of emphasizing gluconeogenesis rather than oxidation appears novel relative to that in mammals. We have no reason to believe that *Dipsosaurus* should be different from other reptiles in its pattern of lactate and glycogen metabolism. Its locomotor capacity, aerobic scope, pattern of gas exchange and lactate accumulation during exercise are all typical of other reptiles and of many other ectothermic vertebrates (Bennett, 1980; Gleeson & Bennett, 1982; Bennett & Dawson, 1972; John-Alder, 1984).

Dipsosaurus dorsalis does not appear to be as heavily dependent on muscle glycogen during exercise as other ectothermic vertebrates: whole-animal glycogen stores are not significantly reduced following activity, although hindlimb musculature shows a significant reduction (Table 1). This is in contrast to the small insectivorous lizard *Sceloporus occidentalis,* in which carcass as well as hindlimb glycogen stores are depleted following 2 min of activity (Gleeson, 1982). Hindlimb glycogen stores were reduced by only about 30 % in *Dipsosaurus dorsalis,* whereas depletion of 60 % or more during brief exercise is common in other vertebrates (Gratz & Hutchison, 1977; Putnam, 1979; Gaesser & Brooks, 1980; Gleeson, 1982; Milligan & Wood, 1986; Peters-Futre *et al.* 1987). The explanation for reduced reliance on glycogen in *Dipsosaurus* is unknown. Regardless of the explanation, it is clear that *Dipsosaurus dorsalis* is not unusually dependent on glycogen metabolism during activity and it is, therefore, unlikely that its pattern of lactate removal during recovery is unusually biased towards pathways of glycogen replenishment relative to that of other vertebrates.

Over 120 min of recovery, 63·1 mg (700 μmol) of lactate were removed from an

average animal of 50 g mass. This was accompanied by the oxidation of 17·1 mg of lactate and the net synthesis of 13·5 mg of glucose and 38·4 mg of hindlimb glycogen (totaling 69·0 mg). The stoichiometry alone suggests that significant muscle glycogenesis occurs during recovery in fasted *Dipsosaurus dorsalis*. Muscle glycogen replenishment after exercise also occurs in fasted fish (Batty & Wardle, 1979; Milligan & Wood, 1986) and in other reptiles (Gratz & Hutchison, 1977; Gleeson, 1982). Modest glycogen replacement has also been shown in nonfasted rats (Fell *et al.* 1980) but not in fasted rats (Brooks *et al.* 1973; Gaesser & Brooks, 1980).

During the first 60 min of recovery, net removal of lactate amounted to 38·3 mg (425 μmol), while 34·3 mg of glycogen was synthesized in hindlimb musculature alone. An additional 24·3 mg of glucose was also synthesized, based on changes in whole-animal glucose concentrations (Table 1). The elevated level of hepatic glucose measured at 30 min (Fig. 2) suggests that hepatic glycogenolysis may account for the discrepancy between the mass of lactate metabolized and the amount of glucose and glycogen synthesized at 60 min. *Dipsosaurus* experience a 26-fold increase in plasma epinephrine following exercise (Dalessio *et al.* 1989), which should stimulate hepatic glycogenolysis, and post-exercise hepatic glycogen depletion is known to occur in other lizards, and in snakes and rats (Brooks *et al.* 1973; Gratz & Hutchison, 1977; Fell *et al.* 1980; Gleeson, 1982). These factors all suggest that hepatic glycogenolysis does contribute to the post-exercise glucose pool in *Dipsosaurus dorsalis*. The magnitude of hepatic glycogenolysis, however, cannot be estimated in *Dipsosaurus* from these data because of variability in hepatic glycogen stores among animals (Table 1). Hepatic glycogen stores were quite high during this study, as occurs seasonally in other iguanid lizards (Gleeson, 1982). The magnitude of the glycogen depletion necessary to account for the net gluconeogenesis not accounted for by lactate catabolism during the first 60 min of recovery is approximately 12·2 mg glycogen g^{-1} liver (approx. 75 mmol kg^{-1}), an amount approximately equal to one standard error of the mean glycogen value at each time interval (Table 1) and, therefore, could be easily concealed within the intraspecific variability. Only remeasurement during a season when hepatic glycogen levels are more constant would resolve this uncertainty.

Pathways of lactate removal

The isotopic data allow us to evaluate the fate of the post-exercise lactate in *Dipsosaurus dorsalis*. Vertebrate tissues have several avenues by which post-exercise lactate can be removed. A predominant fate in mammals is oxidation to CO_2. This CO_2 can in turn enter the tissue bicarbonate pools, or be released as expiratory CO_2. Lactate can also be used to form glucose in tissues endowed with the enzymes necessary to bypass the non-reversible steps of glycolysis (Newsholme & Start, 1973; Connett, 1979). The newly formed glucose can then be stored as tissue glycogen or enter catabolic pathways. Another route of lactate removal involves transamination of pyruvate to alanine (Felig & Wahren, 1973) and incorporation into proteins.

All these pathways are active to some extent, and the multiplicity of fates makes analysis difficult. Another difficulty is that the labeled carbons of lactate may be exchanged with unlabeled carbons as the lactate molecule proceeds along a pathway which shares intermediates with other pathways. This limitation has been addressed by Hetenyi (1982), Katz (1986) and others for the case where ^{14}C is lost from the gluconeogenic pathway as lactate carbon passes through the oxaloacetate pool. It has been shown that unlabeled carbon can replace labeled carbon in the carboxylation/decarboxylation reactions as pyruvate is converted to phosphoenolpyruvate. This exchange can result in an overestimate of the fraction of the lactate pool removed oxidatively. Correction factors have been proposed and used (Hetenyi, 1982) to minimize this error. However, Stevenson *et al.* (1987) have argued that in the case of muscle, gluconeogenesis proceeds by reversal of the pyruvate kinase step, making carbon exchange unlikely and correction unnecessary. A related complication, recently discussed by Sahlin (1987), stems from the fact that the lactate pool is in equilibrium with the pyruvate pool through the enzyme lactate dehydrogenase (LDH) (Wolfe *et al.* 1988). The near equilibrium of LDH means that label will equilibrate in the lactate and pyruvate pools and can be 'washed out' through the pyruvate pool if carbon flux through the pyruvate pool is high relative to the net flux into the pool from lactate. The significance of this process in estimating rates of lactate turnover has been debated (Stanley & Brooks, 1987; Sahlin, 1987), as it can potentially lead to overestimation of lactate turnover and of the fraction of the lactate pool removed by oxidation. This phenomenon may be a significant problem at rest, when lactate turnover rates are low. Stanley & Lehman (1988), however, have modeled this phenomenon and argue that when the rate of net lactate conversion to pyruvate is large relative to the rate of *de novo* pyruvate synthesis, the error in estimating lactate turnover is small. We believe that this is also the case in recovering *Dipsosaurus,* when the rate of net flux through the pyruvate pool from the lactate pool is high. In fact, the differential rates of glucose and lactate oxidation during recovery (see Table 3), suggest that the flux into the pyruvate pool from lactate is approximately 10 times that from phosphoenolpyruvate. Although these shortcomings may limit the utility of using isotopically labeled carbon distributions to estimate lactate turnover or flux quantitatively, they do not seriously compromise our qualitative evaluation of the strategy of lactate removal in this lower vertebrate. In the discussion which follows, we identify these shortcomings as they may apply.

Fate of lactate

After 2 h of recovery, 79 % of the elevated whole-body lactate was removed. The remaining lactate pool retained 24 % of the injected [^{14}C]lactate label (Fig. 4). Subject to the limitations outlined above, the distribution of the remaining 76 % of the label no longer in the lactate pool approximates the fate of the lactate metabolized during the recovery period. Such an interpretation would conclude that after 2 h of recovery, about 46 % of the lactate metabolized has been incorporated into carbohydrate as either tissue glycogen or tissue glucose (Fig. 4).

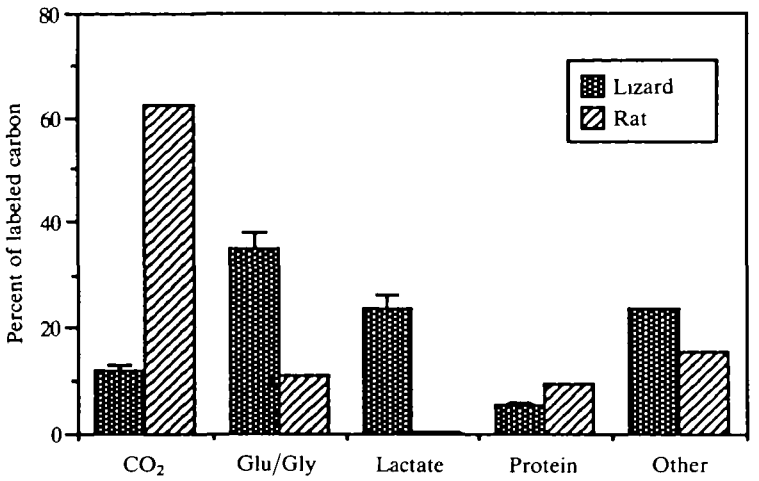

Fig. 5. A comparison of lactate carbon distribution 2 h after exhaustive exercise in the lizard *Dipsosaurus dorsalis* and in the rat, illustrating the different emphasis on oxidative and gluconeogenic removal of lactate in the two animals. CO_2 column combines label in both CO_2 and HCO_3^-, glu/gly combines distribution in both glucose and glycogen. Error bars equal 1 S.E.M. Rat data from table 3 of Brooks & Gaesser (1980).

In contrast to this gluconeogenic fate, 16 % of the lactate carbon was apparently oxidized and recovered as either tissue bicarbonate (2 %) or expiratory CO_2 (14 %). An additional 7 % of the lactate carbon was incorporated into protein.

The fate of lactate varied slightly over the 120 min period. After 1 h, lactate removal *via* gluconeogenesis accounted for nearly 50 % of the metabolized lactate, while oxidation was the fate of an additional 11 % and protein incorporation 5·5 %. The remaining 30 % of the metabolized lactate carbon not accounted for at 60 and 120 min was presumably incorporated into intermediates not measured in this study. Brooks & Gaesser (1980) have identified these endproducts in rats as including alanine, glutamine and a number of tricarboxylic acid cycle (TCA) intermediates.

The pattern of lactate removal in lizards appears to be significantly different from that reported for mammals. The most direct comparison can be made with data from the white rat, where experimental design is most similar to the present study (Fig. 5). In the rat, approximately 60–75 % of the lactate is oxidized during a 2 h recovery period, while approximately 11 % is gluconeogenically converted to glucose or glycogen (Brooks *et al.* 1973; Brooks & Gaesser, 1980). In running dogs 55–75 % of the lactate is estimated to be removed oxidatively, while 10–25 % is gluconeogenically converted to glucose (Depocas *et al.* 1969; Issekutz *et al.* 1976). Although one study in man has estimated lactate conversion to glycogen as the pathway for removal of 75 % of the post-exercise lactate load (Hermansen & Vaage, 1977), another study has concluded that no more than 18 % of post

exercise lactate is removed by this path (Peters-Futre *et al.* 1987). This latter study is in agreement with other studies that conclude that lactate oxidation is the primary pathway for lactate removal during exercise in man (Jorfeldt, 1970; Mazzeo *et al.* 1986; Stanley *et al.* 1988). Although the case in man may not be precisely understood, most mammalian studies suggest an oxidative strategy for lactate removal during and after exercise, a pattern notably different from that reported here for this small lizard.

Lactate metabolism in this reptile also differs from that found in mammals regarding the time course of lactate removal. Although accumulation of lactate in the blood following exercise is similar in magnitude in the two groups, reptiles require a much longer time to remove the accumulated lactate than do mammals. *Dipsosaurus dorsalis* and other reptiles require 2 h or longer to remove a post-exercise lactate accumulation (Fig. 2; Gratz & Hutchison, 1977; Gleeson, 1982), but lactate removal is often complete within 30 min in mammals (Brooks *et al.* 1973). Lactate removal is a slow process in most lower vertebrates, taking 24 h in some fishes (Milligan & Wood, 1986). While it is possible that the slow rate of removal is due to an emphasis on gluconeogenic pathways, it may also be due to the low rate of oxidative metabolism in these animals, which is generally one-sixth to one-eighth that of a mammal or bird of comparable size and body temperature.

Distribution of ^{14}C indicates that 45–50 % of the lactate metabolized during recovery is used as a gluconeogenic substrate. Some authors have argued that simple distribution data underestimate the extent of lactate-derived gluconeogenesis because some of the label is washed out of the gluconeogenic pathway by exchange with unlabeled carbon as lactate carbon passes through the oxaloacetate pool (Hetenyi, 1982; Katz, 1986). Use of correction factors suggested for intact mammals (Hetenyi, 1982) would reduce our estimate of the fraction of lactate oxidized to 2 % or less, while increasing the fraction of metabolized lactate serving as a gluconeogenic substrate to 60–65 %. Use of these correction factors, however, assumes that the carbon donor, acetyl-CoA, is essentially free of ^{14}C, an assumption violated to the extent that lactate carbon does pass through acetyl-CoA *en route* to complete oxidation. In preliminary studies where $[^{14}C]HCO_3^-$ was injected, the extent of glucose and glycogen labeling was insignificant, suggesting that little label exchange occurs with the tissue CO_2 pool. For these reasons we have taken the conservative approach and have left our data uncorrected.

Hepatic lactate metabolism

The data suggest a limited role for the liver in the glucogenic removal of lactate following exercise in these animals. When the plasma glucose pool was labeled by injection of [^{14}C]glucose, little label was measured in the blood lactate pool (Table 2), yet significant label was incorporated into hepatic glycogen. This indicates that glycogen synthesis from glucose occurs in the liver. When the plasma lactate pool was labeled by [^{14}C]lactate injection, however, negligible label was incorporated into liver glycogen during the first 90 min (Table 2). These obser-

vations suggest either that the liver does not utilize significant amounts of blood lactate as a gluconeogenic substrate during the early phase of recovery, in which case other gluconeogenic tissues such as renal tissue must be the source of the labeled plasma glucose that does appear following [^{14}C]lactate injection, or that the glucose produced by the liver from lactate is exported immediately and does not exchange with the hepatic glucose destined for glycogen deposition. The first explanation is in contrast to the role assigned to the liver in the traditional Cori cycle for lactate metabolism, which considers the liver as the principal site of lactate conversion to glucose (Cori, 1931; Gaesser & Brooks, 1980). This explanation is also at odds with the 'indirect pathway' for hepatic glycogen synthesis from lactate, shown to be the predominant postprandial pathway for glycogen synthesis in rats (Foster, 1984). The second explanation would be consistent with the proposal of hepatic compartmentalization of glucokinase and glucose phosphatase discussed by Newsholme & Start (1973). In short, the role of the reptilian liver in post-exercise metabolism is unclear, and much work will be required to define the conditions under which it utilizes lactate and the hormonal control of this process.

Substrate oxidation during recovery

In the only study where lactate and glucose oxidation have been measured in a resting lizard, Guppy *et al.* (1987) found that glucose oxidation accounted for approximately 8% of the resting metabolic rate of fasted animals at 35°C, while lactate oxidation accounted for less than 1% of the resting $\dot{V}_{O_2}$. Fatty acid oxidation was assumed to account for most of the remaining substrate oxidation. The amount of ^{14}C expired during recovery in *Dipsosaurus dorsalis* allows calculation of comparable data in the post-exercised lizard. Using the amount of ^{14}C measured in respiratory CO_2 and tissue bicarbonate, and carcass glucose and lactate specific activities, oxidation rates were calculated assuming 134·4 and 67·2 ml O_2 consumed per mmol glucose and lactate, respectively. These calculations, summarized in Table 3, indicate that approximately 40% of the post-exercise $\dot{V}_{O_2}$ is due to lactate oxidation, while glucose oxidation accounts for only a

Table 3. *Contribution of lactate and glucose oxidation to total* $\dot{V}_{O_2}$ *following exercise in* Dipsosaurus dorsalis

Interval (min)	Animal $\dot{V}_{O_2}$ (ml O_2 g^{-1} h^{-1})	μmol oxidized		% $\dot{V}_{O_2}$ (lactate)	% $\dot{V}_{O_2}$ (glucose)
		Lactate	Glucose		
0–30	0·342	52·2	2·7	40·9	4·16
30–60	0·309	55·6	1·55	48·4	2·70
60–90	0·272	41·4	0·85	40·9	1·68
90–120	0·241	43·8	0·85	48·9	1·90

Calculations are based on animal mass of 50 g, oxidation ratios of 134·4 and 67·2 ml O_2 $mmol^{-1}$ glucose or lactate, respectively.

few percent of the recovery $\dot{V}_{O_2}$. This calculation suggests that the rate of *de novo* carbon flux into the pyruvate pool from glucose is low relative to the net rate of carbon flux from the lactate pool. If this is valid, it indicates that label washout through the pyruvate pool, as suggested by Sahlin (1987), is not of major consequence under these conditions.

Our data do not indicate how the 21% of the lactate accumulated during exercise still remaining after 2 h of recovery is metabolized. Label distribution suggests that more of the lactate will be oxidized after 2 h than after 1 h, but the data cannot be extrapolated to later times with confidence. There is evidence from amphibian muscle that the distribution of lactate carbon to oxidative and gluconeogenic pathways is concentration-dependent (Bendall & Taylor, 1970; Connett, 1979). If the same is true in *Dipsosaurus dorsalis*, we would expect that a progressively greater fraction of the remaining lactate would be oxidized to CO_2 as tissue and plasma lactate concentrations fall during the latter hours of recovery.

In summary, the evidence points to an important role for lactate in the recovery of resting muscle glycogen levels in the small iguanid lizard *Dipsosaurus dorsalis*. Two hours of quiet recovery is accompanied by removal of 79% of the lactate which accumulated during activity, and full replacement of muscle glycogen stores to pre-exercise levels. The isotopic data conservatively estimate that approximately 50% of the lactate removed during the recovery period is used to synthesize glucose and glycogen, while only a minor portion is oxidized. Although the same pathways for lactate removal exist in higher vertebrates the lizard's strategy of utilizing lactate for glycogen resynthesis differs from that of mammals. The strategy seems to place priority on restoring muscle glycogen stores, regardless of time and energetic considerations. Recovery which takes 2 h or more in *Dipsosaurus* is complete in 15 min or less in similar-sized mammals (Brooks *et al.* 1973) and, at a cost of about 8 mol ATP per mol of glycogen glucose synthesized from lactate, it is a costly undertaking. Most iguanid lizards have limited abilities to sustain activity aerobically and, therefore, are more dependent on glycogen stores to support anaerobically fueled predator avoidance, territorial defense and prey-capture activities. For this class of vertebrates, which does not store caches of food, it may be more adaptive to retain most of the lactate to resynthesize glycogen and support the process through the oxidation of non-carbohydrate substrates and some lactate, then to attempt foraging or prey-capture activities when anaerobic support for activity is curtailed by depleted glycogen levels.

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